May 23, I found one strong plant of Astragalus lotiflorus nebraskensis Bates, the only one found within 30 miles. It was well covered with Uromyces astragali, which heretofore has been confined in Nebraska to Astragalus mollissimus and adsurgens. It is common on the former 30 miles west, but I have not found it on that host here, though the host is abundant. I took leaves of this, and laid them in among the leaves of A. shortianus May 28, and later, with no infection. May 29, I did the same to A plattensis and A. crassicarpus, a mile away. June 14, both plants were well infected. A. plattensis however carried on the disease through the season with more vigor, proving the better host.

Red Cloud, Neb., March 7, 1905.

SOME SUGGESTIONS FROM THE STUDY OF DAIRY FUNGI.*

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The importance of certain saprophytic fungi in the arts has only begun to be realized in recent years. Nevertheless numerous papers chiefly chemical have already dealt with the effects of such organisms upon many organic media. It is when one is confronted with one of these problems as a practical proposition and tries to utilize the results of mycological work already published, that he begins to realize the hopeless muddle of our present nomenclature and descriptions in certain cosmopolitan genera.

In the beginning of the dairy investigation with which I am connected I encountered several problems. Nearly all the studies upon milk and milk products have been the work of bacteriologists, consequently when dairy fungi have been concerned these studies are almost purely physiological. In fact in very few cases has sufficient attention been given to the morphology of the forms studied to make their identity in any degree certain. Similarly a number of chemical investigators have studied the effects of fungi upon special media without proper studies of morphology. Thus we find a considerable mass of literature representing a great deal of work in which the species involved can scarcely be determined. So little importance has been attached to species that the author of a recent paper when asked for a culture of his organism replied, that any green Penicillium would produce the same effects. Nevertheless such wide divergence of results as we find in papers dealing with organisms for which the same specific name is used indicates that the

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authors are really using quite different species. If some one were to collect all the effects ascribed to Penicillium glaucum he would be convinced that it was the most versatile organism on earth. But a considerable acquaintance with green Penicilliums leads to the reply that the real organisms involved would make a most motley collection.

In the first place it is perhaps needless to say that most dairy fungi are cosmopolitan saprophytic species. In addition to these any saprophyte or even parasite capable of growing in milk or common culture media may be found there at times. Although this latter group may be numerous at a particular time and place they are comparatively easily distinguished from that special lot of species which are almost universal in dairy cultures. Here we encounter a great difficulty. Mycological literature is full of descriptions of saprophytes such as Penicillium, Cladosporium, Aspergillus, Fusarium, Alternaria, and worse still Monilia and Oospora (Oidium) in which brief notes upon a single colony of fungus upon a named substratum are the only basis for identification. In these one feels fortunate when he finds a spore measurement. Too commonly the substratum named is either unique or very vaguely defined, while the suggestion of culture is a rarity. Satisfactory identification a second time by another worker might occur, but only accidentally.

Dr. Fr. Dierckx in speaking of the genus Penicillium (translating freely), says, "For the genus Penicillium it is only exceptionally possible to identify a type met by accident upon accidental substratum, without cultivation. In fact the molds vary immensely according to the circumstances under which they are observed." He continues saying that in such a genus the only hope for stable identification is in culture for several generations under "rigorously uniform conditions."

This paper attempts to present a plan for obtaining more definite knowledge of these forms by the dairy student in the use of his own methods. The media used have been the nutrient agar and peptone milk-sugar-gelatine described by Conn (Bact. in Milk and its Products p. 253, 268) in addition to ordinary potato agar and potato plugs. Special experiments have involved many other media. These cultures were commonly acidified by two to five drops of normal lactic acid to exclude bacterial growth. Once freed from bacteria the fungi were studied in both acid and neutral media.

It is useless to say that any classification of such forms as Penicillium and Aspergillus, to be of value, must rest upon ascus formation. The discovery of such fruit or methods of inducing its formation in particular species is of much interest, but since the vast majority of the species concerned have failed to produce asci under stress of thousands of cultures by numberless

students it becomes economically necessary to find some means of satisfactorily describing the forms we have.

The method of identifying by hosts or substrata has wrought endless confusion. Many of these species are cosmopolitan and omnivorous in their conidial or hyphomycete form. Not only so, but they present morphological adaptations to different substrata. I cannot agree with the author who recently described a Fusarium as new, with the plea that the possibility of finding it already described involved much labor and anyway (translating) "Just as many species of Fusarium are made as there are host plants found infested with Fusarium." Such a study to be of value to-day must present both morphology and physiological effect under known and easily reproduced conditions.

But since my specific problem is cultural, and since the importance of these organisms is due to their constant presence in all cultural work, in the dairy, in the household, in every form of manufacture which offers food material to omnivorous organisms, I set out the definite task of finding to what extent it is possible to find reliable diagnostic characters in their relations to standard culture media. In the discussion I will draw my suggestions from comparative studies of some fifteen species of Penicillium.

Every Penicillium appearing in hundreds of plates in two dairy bacteriological laboratories was isolated and studied. Many more have been secured from distant laboratories. Series of petri-dish cultures representing every form found have been made and constantly watched for diagnostic characters. Easily confused forms have been grown in parallel cultures for generation after generation that such differences as appeared should be fully tested. The same species has been cultivated on gelatine, agar, potato, cheese, milk, caseine, manure, wood, Raulin's fluid, and other special media. Stimulation by acids and alkalies and by the excretions of other fungi and bacteria has been tried. In spite of all the changes in morphology and effect, due to such treatment, a return to the original gelatine has in most cases brought with it a remarkably constant return to the same structures and effects as upon the plates first thoroughly studied. In cases of special difficulty several forms are carefully inoculated into one cold poured plate and their positions marked and their entire course of development is watched under conditions whose uniformity cannot be questioned. Thus the question what characters may be depended upon as constant under constant conditions, has been kept always in view. This is in direct contrast to those special investigations where the authors have set themselves to the task of inducing and describing variations in particular species. Such studies have commonly given very little attention to the problem of furnishing adequate means

by which others could be certain they were in possession of the same species. It is manifestly impracticable to repeat a long series of variation experiments with a number of different fungi simply as a means of identification of a form sought. It seems, therefore, very desirable to present the results of an effort to find the simplest means of characterizing species.

In attempting to develop a method of description which will be practicable, I have limited the media used as much as possible. Within this limitation attention is directed to such morphological and physiological characters of the colonies as seem to be of real value. A card on the plan of the bacteriologists' has been prepared for rapid comparison of results. There are abundant weaknesses in this method, but it is intended to call attention to important and easily observed differences between forms, and ample provision is made for the usual complete written description is well. Such description makes necessary the adaptation and definition of a set of terms to avoid confusion, and one must recognize as well that no set of terms was ever completely satisfactory to describe living things. If we remember then that a series of observations must cover the whole growing and fruiting period of the species, perhaps for several generations, we will find some such system as I now propose, suggestive at least.

The characters used may be best examined separately.

I. *Relation to Culture Media.*

1. *Fruiting period.*

In studying a series of cultures it may be very readily shown that under exactly the same conditions two species may develop from the spore to the ripe fruit in very different times. Whether this minimum period is the same or different, its relative length is fairly constant for the two, and this commonly remains true under changed conditions. For example, the Penicillium used in ripening Camembert cheese was first separated from contamination by its much slower development than the entirely worthless species mixed with it. Similarly the length of the period during which spores are produced varies greatly. Some species produce spores from a single set of conidiophores in a very limited period then die — apparently. Others produce a succession of conidiophores from the same mycelium, which requires a much longer period so that the maximum time for different species varies from a few days to several weeks on exactly the same media. Whether the limitation of period is due to definiteness of life cycle or to excretory products which inhibit further growth is unsettled, but the fact of limitation is reasonably clear in many species where the substratum is in no way exhausted.

2. *Gelatine.*

The diagnostic value of the liquefaction of gelatine is questioned by some, but within certain limits I believe it to be very useful. Colonies of some species never produce such liquefaction, other colonies will be floating entirely free in a pool of liquid within a week (ex. Cladosporium herbarum) and always produces such an effect. The character is very reliable as an accessory to other data; in such cases many species produce slight liquefaction, some liquefy the gelatine under the center of the colony, remaining always with at least a sterile vegetative border of mycelium in the solid substratum, some produce a semi-liquid condition in the whole plate but no watery fluid. In these latter cases especially this is difficult to describe accurately and perhaps unreliable because the extent of change is often both variable and dependent upon conditions which cannot be accurately determined. However, this indefinite condition should be described and recorded as carefully as observations will permit. Whether the liquefaction of gelatine is to be regarded as an index of enzyme production will be discussed in another paper, but the question is entirely too indefinitely understood to give it a diagnostic value as great as some bacteriologists assume it to have. Further, it must be noted here that the same species gives different reactions to gelatine media of different formulae so that much care is necessary in stating results of this kind.

3. *Indicators.*

The introduction of a sterile solution of litmus or other indicator gives many interesting contrasts. With litmus in media acidified by two to five drops of normal lactic acid, cultures of some species uniformly show a prompt and sharp change from red to blue, beginning as soon as the growth becomes visible to the eye. Cultures of other species show no change for several days, the time here being fairly characteristic, then with the change of color in the fruiting portion of the mycelium (or about that time) the medium begins to turn blue below the center of the colony and this color progresses outward until usually the entire plate or tube of medium has become blue. In still other species a red medium remains red during its entire growing, and fruiting period. If the medium be neutral or alkaline those which turn red to blue will leave the blue entirely unchanged, others will change the blue to red for a time varying with the species after which it progressively changes back to blue, beginning at the center as before; others will turn the blue to red and it will remain so. Similarly here we find every gradation in rate and intensity of action which brings into notice some species whose effect is neutral, giving the shades of color characteristic of the turning point of litmus and other species in which discordant results are obtained from causes not yet determinable. Such

difficulties are special cases and do not destroy the significance of the definite conduct of very many species. The value of other indicators has not been tested sufficiently to report results. Very few of the species tested so far, carry the medium to the alkaline side of Phenolphthalein, though several of the more strongly alkaline forms have done this.

4. *Secretions.*

Pigment production.— Some species produce pigments which are secreted, or perhaps better, excreted, into the substratum. Among the species of Penicillium studied at least two produce bright yellow pigment in considerable quantity. Several more at times exhibit a trace of this ability. Pigment production is dependent apparently upon the substratum. Neither of these species produce any color within potato agar but both will rapidly turn a tube of milk yellow or cause large patches of yellow on bread, or potato plugs. This yellow pigment is soluble in alcohol. The whole question of pigments is, however, so imperfectly known that its diagnostic value is very doubtful except in a few cases.

II. *The Colony Itself.*

Confronted with the necessity of being able to distinguish certain species, I next turned to the colonies and sought for characters which might be of value. Not to review failures certain points have proved to be fairly reliable. Approximate uniformity of media must be kept in mind, but the ordinary differences between successive lots of gelatine or agar upon the same formula do not seem to disturb these relations in the species I have studied. I am informed by Dr. Haven Metcalf of Clemson College that the slightest changes in the medium do produce striking morphological changes in certain species of Frusarium.

1. *Color.*

The color and color-changes of colonies though difficult to describe must be observed and recorded for the entire growing period. In spite of the haziness of nomenclature in greens, blue greens, greys and browns, general distinctions can be made and are fairly reliable. Improvement in color charts would aid matters greatly. Milburn has recently shown that changes in acidity of media in certain cases cause radical changes in the color of spores, and similarly variations in illumination and osmotic pressure affect the pigments in the spores of Hypocrea. Although my experiments with Penicillium have not yet produced such results this observation has been made upon P. glaucum by Dr. O. Stoll in a recent paper. This makes thorough knowledge and statement of conditions very necessary to uniformity of results.

2. *Surface.*

The comparison of hundreds of cultures of familiar species shows that the general appearance or texture of the adult colony is fairly stable. I have designated this in my description card as the "surface" of the colony. In comparing such surfaces two general types are seen: one, those in which every conidiophore or the vast majority of conidiophores in the growing portion of the colony arises directly from the submerged mycelium through the culture media; the other, those in which the conidiophores are produced as lateral branches of matted aerial hyphae. The first type results as a rule in a colony which lies very close to the substratum without loose networks of hyphae and may be designated as *"strict;"* the second, a piling up of masses of loose hyphae which may be called *"floccose."* A strict surface may be *close* where the fructifications are barely elevated above the medium making a smooth area or *lax* if the conidiophores are longer and give a velvety appearance. Floccose surfaces may be loose or closely woven, felted or tufted, stilboid (bearing large coremia), stolon bearing or composed of divergent ropes of hyphae or aerial hyphae may be so sparingly developed as to appear *strict.* Such a colony might be styled *appressedly floccose* or *falsely strict.*

3. *Margin.*

In discussing the surface of the colony the fact was brought out that the borders of the rapidly growing colony show much more uniformity than the center which has ceased growing. Study of the margin in the old colony is usually valueless, but during rapid growth it shows how the fungus spreads in the substratum, the branching, septation and measurements of the hyphae, and the origin of all aerial structures. One has for example but to study the growing border of a single colony of the common Oidium (Oospora) lactis to recognize its peculiar dichotomous branching, anywhere afterward. Two types here will include by far the larger number of species. One may be called "indeterminate." In this group the submerged vegetative mycelium forms a distinct band beyond the aerial and fruiting portion. In the other for which the term "determinate" is much less accurate, the aerial portion travels or appears on the surface as fast as the vegetative mycelium spreads in the substratum. An indeterminate or diffuse colony will show a succession of fruiting branches from conidiophores with ripe fruit in the center, to delicate rudiments just breaking singly through the surface at the margin. Such a colony will show a marked tendency to spread all over whatever surface is offered to it. A determinate colony is usually restricted in growth. Fully-formed fruit will often be found at the very edge if the colony is strict, with a very

narrow border of white if any appears. In a floccose colony loose aerial hyphae with or without fruit will spread as fast in the rapidly growing period as the vegetative hyphae. Later this balance is destroyed in some species so that they appear almost as if indeterminate.

4. *Conidiophore.*

The conidiophores are a variable quantity. There is, however, a general type or series of types in each form which is found of value. The length of the conidiophores, their septation, the diameter of their cells, and especially their origin and relation to the substratum and to each other are sufficiently characteristic to be very useful.

5. *Fructification.*

The term fructification may be best made to include the chains of conidia and the basidia and branches bearing them back to the first branch from the main conidiophore. Such fructifications are variable and exceedingly troublesome to figure satisfactorily. The data which are offered vary with the species, but include the mode of branching, the measurements of the basidia, the relation of the branches and basidia to each other, the collocation of the chains of conidia, and, perhaps the most useful of all, measurements of the limits of total length and breadth of the whole fructification. In some species the chains are widely divergent, in others joined into a column. In some all the basidia are in a single verticil, such a fructification may be called *simple.* In others the first branch is divergent giving a *falsely double* effect. A conventionalized diagram of a series of fructifications enlarged about 100 diameters and sketched under the camera-lucida shows very striking contrasts between different species.

6. *Conidia.*

With reference to the conidia there is no departure from the usual data,—size, color, presence or absence of a connective, mode of germination, markings on surface, tendency to adhere or separate freely, rapidity of growth and resistance to destructive agents or conditions.

A long series of cultures indicates that for the genus Penicillium at least these characters form a practicable basis for description and it seems reasonable to believe from comparative cultures already made that the same plan could have much wider application.

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